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(54) Title: SELF-ASSEMBLING RECOMBINANT PAPILLOMAVIRUS CAPSID PROTEINS		
(57) Abstract Recombinant papillomavirus capsid proteins that are capable of self-assembly into capsomer structures and viral capsids that comprise conformational antigenic epitopes are provided. The capsomer structures and viral capsids, consisting of the capsid proteins that are expression products of a bovine, monkey or human papillomavirus L1 conformational coding sequence proteins, can be prepared as vaccines to induce a high-titer neutralizing antibody response in vertebrate animals. The self assembling capsid proteins can also be used as elements of diagnostic immunoassay procedures for papillomavirus infection.		

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SELF-ASSEMBLING RECOMBINANT PAPILLOMAVIRUS CAPSID PROTEINS

Field of the Invention

5 This invention relates to recombinant viral proteins. It relates particularly to recombinant viral proteins that are suitable for use in the diagnosis, prophylaxis and therapy of viral infections.

Background of the Invention

10 Papillomaviruses infect the epithelia of a wide variety of species of animals, including humans, generally inducing benign epithelial and fibro-epithelial tumors, or warts, at the site of infection. Each species of vertebrate is infected by a distinct group of papillomaviruses, each papillomavirus group comprising several papillomavirus types. For example, more than 60 different human papillomavirus (HPV) genotypes have been isolated. Papillomaviruses are highly species specific infective agents; for example, a bovine papillomavirus cannot
15 induce papillomas in a heterologous species, such as humans. Papillomavirus types ALSO appear to be highly specific as immunogens in that a neutralizing immunity to infection against one papillomavirus type does not usually confer immunity against another type, even when the types infect an homologous species.

 In humans, genital warts, which are caused by human papillomaviruses, represent a
20 sexually transmitted disease. Genital warts are very common, and subclinical, or inapparent HPV infection is even more common than clinical infection. Some benign lesions in humans, particularly those arising from certain papillomavirus types, undergo malignant progression. For that reason, infection by one of the malignancy associated papilloma virus types is considered one of the most significant risk factors in the development of cervical cancer, the
25 second most common cancer of women worldwide (zur Hausen, H., 1991; Schiffman, M. 1992). Several different HPV genotypes have been found in cervical cancer, with HPV16 being the most common type that is isolated from 50% of cervical cancers.

 Immunological studies demonstrating the production of neutralizing antibodies to papillomavirus antigens indicate that papillomavirus infections and malignancies associated
30 with these infections in vertebrate animals could be prevented through immunization; however the development of effective papillomavirus vaccines has been impeded by a number of difficulties.

 First, it has not been possible to generate in vitro the large stocks of infectious virus required to determine the structural and immunogenic features of papillomavirus that are
35 fundamental to the development of effective vaccines. Cultured cells express papillomavirus

-2-

oncoproteins and other non-structural proteins and these have been extensively studied in vitro; but expression of the structural viral proteins, L1 and L2 (and the subsequent assembly of infectious virus) occurs only in terminally differentiated layers of infected epithelial tissues. Therefore, the characterization of viral genes, proteins, and structure has necessarily been assembled from studies of virus harvested from papillomas. In particular, papillomavirus structure and related immunity have been carried out in the bovine papillomavirus system because large amounts of infectious virus particles can be isolated from bovine papillomavirus (BPV) warts.

The information derived from studies of papillomavirus structure to date indicates that all papillomaviruses are non-enveloped 50-60 nm icosahedral structures (Crawford, L., et al., 1963) which are comprised of conserved L1 major capsid protein and less well conserved L2 minor capsid protein (Baker, C., 1987). There is no sequence relationship between the two proteins. The function and location of L2 in the capsid is unclear; however immunologic data suggests that most of L2 is internal to L1.

Recently, high resolution cryoelectron microscopic analysis of BPV1 and HPV1 virions has determined that the two viruses have a very similar structure, with 72 pentameric capsomers, each capsomer presumably composed of five L1 molecules, forming a virion shell with T=7 symmetry (Baker, T., 1991). The location of the minor L2 capsid protein in the virion has not been determined, and it is not certain whether L2 or other viral proteins are needed for capsid assembly. Superficially, papillomavirus structure resembles that of the polyoma 45 nm virion, which has the same symmetry and capsomere number (Liddington, R., et al., 1991); however, the systems of intracapsomer contact for polyomavirus and papillomavirus species are different, and the major and minor capsid proteins of polyomavirus are not genetically related to L1 and L2.

Bovine papillomavirus studies are facilitated by a quantitative focal transformation infectivity assay developed for BPV that is not available for HPV (Dvoretzky, I., et al., 1980), and an understanding of immunity to papillomavirus has therefore also been derived from the bovine papillomavirus system. Limited studies using intact bovine papillomavirus demonstrated that the non-cutaneous inoculation of infectious or formalin-inactivated BPV virus was effective as a vaccine to prevent experimental BPV infection in calves (Olson, C., et al., 1960; Jarrett, W., et al., 1990). Unfortunately, BPV virions cannot be used to develop vaccines against papillomavirus which infects other species, or even vaccines against other bovine types, because of the great specificity of these viruses, as well as concern for the oncogenic potential of intact viral particles.

-3-

A significant conclusion of studies of papillomavirus immunity is that the ability of antibodies to neutralize papilloma virus appears to be related to their ability to react with type-specific, conformationally dependent epitopes on the virion surface. For example, rabbit antisera raised against infectious BPV1 virions inhibits focal transformation of C127 cells (Doretzky, I., et al., 1980), as well as the transformation of fetal bovine skin grafts; whereas antisera raised against denatured virions does not (Ghim, S., et al., 1991).

In contrast, neutralizing sera generated against bacterially derived BPV L1 and L2 (Pilacinski, W. et al., 1984; Jin, X., et al., 1989) and against in vitro synthesized cottontail rabbit papillomavirus (CRPV) L1 and L2 (Christensen, N., et al., 1991; Lin, Y-L, et al., 1992), neither of which has the structural features of native virions, had low titers, and the use of recombinant HPV L1 fusion peptides expressed in *E. coli* to detect cellular immune reactivity has had only limited success (Höpfel, R. et al., 1991). The results in the BPV system are consistent with those of the HPV system, in which monoclonal antibodies that neutralized HPV11 infection in a mouse xenograft assay recognized native, but not denatured, HPV11 virions (Christensen, N., et al., 1990).

There have been isolated attempts to produce papillomavirus capsids in vitro. Zhou, J. et al. (1991) and (1992) produced virus-like particles by cloning HPV L1 and L2 genes, and HPV L1 and L2 genes in combination with HPV E3/E4 genes into a vaccinia virus vector and infecting CV-1 mammalian cells with the recombinant vaccinia virus. These studies were interpreted by Zhou to establish that expression of HPV16 L1 and L2 proteins in epithelial cells is necessary and sufficient to allow assembly of virion type particles. Cells infected with doubly recombinant vaccinia virus which expressed L1 and L2 proteins showed small (40 nm) virus-like particles in the nucleus that appeared to be incompletely assembled arrays of HPV capsomers. Expressing L1 protein alone, or L2 protein alone, was expressed did not produce virus-like particles; cells doubly infected with singly recombinant vaccinia virus containing L1 and L2 genes also did not produce particles. No neutralizing activity was reported.

Ghim et al., (1992) reported that when L1 from HPV1, a non-genital virus type associated mainly with warts on the hands and feet, was expressed in mammalian cells, the L1 protein contained conformational epitopes found on intact virions. Ghim did not determine if particles were produced, nor was it evaluated if the L1 protein might induce neutralizing antibodies. Even more recently, Hagansee, et al. (1993) reported that when L1 from HPV1 was expressed in human cells, it self-assembled into virus-like particles. No neutralizing antibody studies were performed.

-4-

Studies in other virus systems, for example, parvovirus, indicate that capsid assembly alone may not confer immunogenicity. Parvovirus VP2, by itself, was able to self-assemble when expressed in insect cells, but only particles containing both VP1 and VP2 were able to induce neutralizing antibodies (Kajigaya, S., et al., 1991).

5 It would be advantageous to develop methods for producing renewable papillomavirus reagents of any selected species and type in cell culture. It would also be beneficial to produce such papillomavirus reagents having the immunity conferring properties of the conformed native virus particles that could be used as a subunit vaccine.

10 It is therefore the object of the invention to provide these recombinant conformed papillomavirus proteins, as well as methods for their production and use.

Summary of the Invention

The invention is directed to the diagnosis and prevention of papillomavirus infections and their benign and malignant sequelae by providing recombinant papillomavirus capsid proteins that self assemble to form capsomer structures comprising conformational epitopes that are highly specific and highly immunogenic. Therefore, according to the invention there is provided a genetic construct, comprising a papillomavirus L1 conformational coding sequence, inserted into a baculovirus transfer vector, and operatively expressed by a promoter of that vector. The papillomavirus L1 conformational coding sequence can be isolated from a bovine, monkey, or human gene. In a preferred embodiment, the papillomavirus L1 conformational coding sequence is isolated from a wild type HPV16 gene. In a particularly preferred embodiment, the papillomavirus L1 conformational coding sequence is SEQ ID NO:6. The genetic construct can further comprise a papillomavirus L2 coding sequence.

25 According to another aspect of the invention there is provided a non-mammalian eukaryotic host cell transformed by the genetic constructs of the invention.

According to yet another aspect of the invention there is provided a method for producing a recombinant papillomavirus capsid protein, assembled into a capsomer structure or a portion thereof, comprising the steps of (1) cloning a papillomavirus gene that codes for an L1 conformational capsid protein into a transfer vector wherein the open reading frame of said gene is under the control of the promoter of said vector; (2) transferring the recombinant vector into a host cell, wherein the cloned papillomavirus gene expresses the papillomavirus capsid protein; and (3) isolating capsomer structures, comprising the papillomavirus capsid protein, from the host cell. In a preferred embodiment, the cloned papillomavirus gene consists essentially

-5-

of the conformational L1 coding sequence, and the expressed protein assembles into capsomer structures consisting essentially of L1 capsid protein. In another preferred embodiment, the cloning step of the method further comprises the cloning of a papillomavirus gene coding for L2 capsid protein, whereby said L1 and L2 proteins are
5 coexpressed in the host cell, and wherein the isolated capsomer structures comprise L1 and L2 capsid proteins;

provided that said transfer vector is not a vaccinia virus when said host cell is a mammalian cell. The conformational L1 coding sequence can be cloned from a bovine, monkey, or human papillomavirus. According to a preferred embodiment, the conformational L1 coding
10 sequence is cloned from a wild type HPV16 papillomavirus. In a particularly preferred embodiment, the conformational L1 coding sequence is SEQ ID NO:6. Also in a preferred embodiment, the host cell into which the genetic construct is transfected is an insect cell. Also preferred are embodiments wherein the transfer vector is a baculovirus based transfer vector, and the papillomavirus gene is under the control of a promoter that is active in
15 insect cells. Accordingly in this embodiment, the recombinant baculovirus DNA is transfected into Sf-9 insect cells, preferably co-transfected with wild-type baculovirus DNA into Sf-9 insect cells.

In an alternative embodiment of the method of the invention, the transfer vector is a yeast transfer vector, and the recombinant vector is transfected into yeast cells.

20 According to yet another aspect of the invention there is provided a virus capsomer structure, or a portion thereof, consisting essentially of papillomavirus L1 capsid protein, produced by the method the invention. Alternatively, the virus capsomer structure can consist essentially of papillomavirus L1 and L2 capsid proteins, produced by the method of the invention. In a particularly preferred embodiment, the virus capsomer structure
25 comprises papillomavirus L1 capsid protein that is the expression product of an HPV16 L1 DNA cloned from a wild type virus.

The virus capsids or capsomer structures of the invention, or portions or fragments thereof, can consist essentially of papillomavirus L1 capsid protein. Alternatively, these capsids or capsomer structures or their fragments can consist essentially of wild type HPV16
30 papillomavirus L1 capsid protein.

The virus capsid structures according to any of the methods of the invention comprise capsid proteins having immunogenic conformational epitopes capable of inducing neutralizing antibodies against native papillomavirus. The capsid proteins can be bovine, monkey or human papillomavirus L1 proteins. In a preferred embodiment, the

-6-

papillomavirus L1 capsid protein is the expression product of a wild type HPV16 L1 gene. In a particularly preferred embodiment, the HPV16 L1 gene comprises the sequence of SEQ ID NO:6.

According to yet another aspect of the invention there is provided a unit dose of a vaccine, comprising a peptide having conformational epitopes of a papillomavirus L1 capsid protein, or L1 protein and L2 capsid proteins, in an effective immunogenic concentration sufficient to induce a papillomavirus neutralizing antibody titer of at least about 10^3 when administered according to an immunizing dosage schedule. In a preferred embodiment, the vaccine comprises an L1 capsid protein which is an HPV16 capsid protein. In a particularly preferred embodiment, the vaccine comprises an L1 capsid protein that is a wild type HPV16 L1 protein.

Use of the L1 open reading frame (ORF) from a wild type HPV16 papillomavirus genome, according to the methods of the invention, particularly facilitates the production of preparative amounts of virus-like particles on a scale suitable for vaccine use.

According to yet another aspect of the invention, there is provided a method of preventing or treating papillomavirus infection in a vertebrate, comprising the administration of a papillomavirus capsomer structure or a fragment thereof according to the invention to a vertebrate, according to an immunity-producing regimen. In a preferred embodiment, the papillomavirus capsomer structure comprises wild type HPV16 L1 capsid protein.

The invention further provides a method of preventing or treating papillomavirus infection in a vertebrate, comprising the administration of the papillomavirus capsomer structure of the invention, or a vaccine product comprising the capsomer structure to a vertebrate, according to an immunity-producing regimen. In a preferred embodiment, the papillomavirus vaccine comprises wild type HPV16 L1 capsid protein.

Also within the scope of the invention is a method for immunizing a vertebrate against papillomavirus infection, comprising administering to the vertebrate a recombinant genetic construct of the invention comprising a conformational papillomavirus L1 coding sequence, and allowing said coding sequence to be expressed in the cells or tissues of said vertebrate, whereby an effective, neutralizing, immune response to papillomavirus is induced. In a preferred embodiment, the conformational papillomavirus L1 coding sequence is derived from human papillomavirus HPV16. In a particularly preferred embodiment, the human papillomavirus HPV16 is a wild type papillomavirus.

According to yet another aspect of the invention, there is provided a method of detecting humoral immunity to papillomavirus infection in a vertebrate comprising the steps

-7-

of: (a) providing an effective antibody-detecting amount of a papillomavirus capsid peptide having at least one conformational epitope of a papillomavirus capsomer structure; (b) contacting the peptide of step (a) with a sample of bodily fluid from a vertebrate to be examined for papillomavirus infection, and allowing papillomavirus antibodies contained in said sample to bind thereto, forming antigen-antibody complexes; (c) separating said complexes from unbound substances; (d) contacting the complexes of step (c) with a detectably labelled immunoglobulin-binding agent; and (e) detecting anti-papillomavirus antibodies in said sample by means of the labelled immunoglobulin-binding agent that binds to said complexes. In a preferred embodiment of this aspect of the invention, the peptide consists essentially of papillomavirus L1 capsid protein. According to an alternative embodiment, the peptide consists essentially of the expression product of a human papillomavirus HPV16. In a particularly preferred embodiment, the peptide consists essentially of the expression product of a wild type human papillomavirus HPV16 gene, for example, the peptide can consist essentially of the expression product of SEQ ID NO:6.

According to yet another aspect of the invention, there is provided a method of detecting papillomavirus in a specimen from an animal suspected of being infected with said virus, comprising contacting the specimen with antibodies having a specificity to one or more conformational epitopes of the capsid of said papillomavirus, wherein the antibodies have a detectable signal producing label, or are attached to a detectably labelled reagent; allowing the antibodies to bind to the papillomavirus; and determining the presence of papillomavirus present in the specimen by means of the detectable label.

According to yet another aspect of the invention, there is provided a method of determining a cellular immune response to papillomavirus in an animal suspected of being infected with the virus, comprising contacting immunocompetent cells of said animal with a recombinant wild type papillomavirus L1 capsid protein, or combined recombinant L1 and L2 capsid proteins according to the invention; and assessing cellular immunity to papillomavirus by means of the proliferative response of said cells to the capsid protein. In a preferred embodiment of this aspect of the invention, the recombinant papillomavirus protein is introduced into the skin of the animal.

According to yet another aspect of the invention there is provided a papillomavirus infection diagnostic kit, comprising capsomer structures consisting essentially of papillomavirus L1 capsid protein, or capsomer structures comprising papillomavirus L1 protein and L2 capsid proteins, or antibodies to either of these capsomer structures, singly

-8-

or in combination, together with materials for carrying out an assay for humoral or cellular immunity against papillomavirus, in a unit package container.

Detailed Description of the Invention

5 We have discovered that the gene coding for the L1 major capsid protein of BPV or HPV16, following introduction into host cells by means of an appropriate transfer vector, can express L1 at high levels, and that the recombinant L1 has the intrinsic capacity to self-assemble into empty capsomer structures that closely resemble those of an intact virion.

10 Further, the self-assembled recombinant L1 capsid protein of the invention, in contrast to L1 protein extracted from recombinant bacteria, or denatured virions, has the efficacy of intact papillomavirus particles in the ability to induce high levels of neutralizing antiserum that can protect against papillomavirus infection. The high level of immunogenicity of the capsid proteins of the invention implies strong antibody binding properties that make them sensitive agents in serological screening tests to detect and measure antibodies to conformational virion epitopes. Their immunogenicity also indicates
15 that the capsid proteins of the invention can also be used as highly effective vaccines or immunogens to elicit neutralizing antibodies to protect a host animal against infection by papillomavirus. These observations were recently published in Kirnbauer, et al., (1992), and formed the basis of U.S. application Serial No. 07/941,371.

20 We have now discovered that the capsid protein L1 expressed by wild type HPV16 genomes isolated from benign papillomavirus lesions, when expressed in the baculovirus system described, will self-assemble with an efficiency heretofore unknown and comparable to that of bovine papillovirus L1 capsid protein.

The HPV16 L1 Gene Sequence

25 The source of HPV16 L1 DNA, as disclosed in published studies, for example, by Zhou, et al.(1991) was the prototype clone, GenBank Accession No. K02718, that had been isolated from a cervical carcinoma (Seedorf, et al., 1985). We have found that L1 from wild type HPV16 genome, which differs from the prototype genome by a single point mutation, will self-assemble into virus-like particles with an efficiency similar to that seen with BPV L1 or BPV L1/L2. Compared with the self-assembly seen when L1 from the prototype
30 HPV genome is used with L2, L1 from a wild-type genome self-assembles at least 100 times more efficiently.

To provide genetic insight into the self-assembly efficiency of different HPV16 L1 expression products, the open reading frames from HPV16 L1 genes isolated from both benign lesions and lesions associated with dysplasia or carcinoma were sequenced.

-9-

The analysis detected two errors in the published sequence of the published L1 sequence of the prototype strain, as follows:

- (1) there should be an insertion of three nucleotides (ATC) between nt 6902 and 6903, which results in the insertion of a serine in the L1 protein; and
- (2) there should be a deletion in the published prototype sequence of three nucleotides (GAT), consisting of nt 6952-6954, which deletes an aspartate from the L1 protein sequence. The corrected nucleotide sequence of the prototype HPV16 L1 genome, consisting of nt 5637-7155, is that of SEQ ID NO:5, listed herein.

The numbering of the nucleotide bases in Sequence ID Nos. 5 and 6 is indexed to 1, and the numbering of nucleotide bases of the published HPV sequence, that is from nt 5638-7156, corresponds to those of the sequence listing from 1-1518. The sites referred to in the original sequence can be thus readily identified by one skilled in the art.

Three other HPV16 L1 genomes, clone 16PAT; and clones 114/16/2 and 114/16/11, were sequenced and those sequences compared to that of the corrected prototype.

Clone 16PAT, kindly provided by Dennis McCance at the University of Rochester School of Medicine, and cloned from a dysplastic (pre-malignant) lesion of the cervix, expresses an L1 that does not self-assemble efficiently.

Clones 114/16/2 and 114/16/11, kindly provided by Matthias Dürst of the German Cancer Research Center in Heidelberg, were both cloned from non-malignant lesions, and both expressed L1 protein that self-assembled efficiently.

Comparison of Genetic Characteristics of HPV16 L1 associated with Dysplasia, Malignant Progression and Benign Lesions

Clone 16PAT, isolated from papillomavirus infected dysplastic lesions and the prototype HPV16, isolated from malignant cervical carcinoma, both encode Histidine at nt 6242-6244, while clones 2 and 11, isolated from benign papillomavirus infected lesions (like isolates of many other papillomavirus) encode Aspartate at this site.

It appears that this single amino acid difference between the prototype, malignancy-associated HPV16 species, and the HPV16 species from benign lesions accounts for the difference in self-assembly efficiency. It is likely that among closely related HPV types, Aspartate at this locus may be necessary for efficient self-assembly, and that the substitution of Histidine for Aspartate impairs this ability in the capsid protein. The impairment in capsid assembly in malignancy-associated viruses, associated with loss of the conformational

-10-

epitopes required for the production of neutralizing antibodies, may also be linked to a lowered immunogenicity which would allow the papillomavirus to escape immune control.

Accordingly, HPV16 L1 genes that express capsid protein that self-assembles efficiently can be obtained by

- 5 (1) isolation of the wild type HPV16 L1 open reading frame from benign lesions of papillomavirus infection; or
- (2) carrying out a site specific mutation in the prototype sequence at nt 6242-6244 to encode Aspartate.

Recombinant Capsid Protein

10 The method of the invention provides a means of preparing recombinant capsid particles for any papillomavirus. Particles consisting of either L1 or L2 capsid protein alone, or consisting of both L1 and L2 capsid proteins together can be prepared. L1/L2 capsid protein particles are more closely related to the composition of native papillomavirus virions, but L2 does not appear to be as significant as L1 in conferring immunity, probably
15 because most of L2 is internal to L1 in the capsid structure. Although L1 can self-assemble by itself, in the absence of L2, self-assembled L1/L2 capsid protein particles are more closely related to the composition of native papillomavirus virions. Accordingly, particles comprising L1 alone are simpler, while those comprising L1/L2 may have an even more authentic structure. Both self-assembled L1 and L1/L2 particles induce high-titer
20 neutralizing antibodies and may therefore be suitable for vaccine production. Particles comprising L1 capsid protein expressed by a wild type HPV genome, either as L1 alone or L1/L2 together, are particularly preferred.

Production of the recombinant L1, or combined L1/L2, capsid particles is carried out by cloning the L1 (or L1 and L2) gene(s) into a suitable vector and expressing the
25 corresponding conformational coding sequences for these proteins in a eukaryotic cell transformed by the vector. It is believed that the ability to form a capsid-like structure is intimately related to the ability of the capsid protein to generate high-titer neutralizing antibody, and that in order to produce a capsid protein that is capable of self-assembling into capsid structures having conformational epitopes, substantially all of the capsid protein
30 coding sequence must be expressed. Accordingly, substantially all of the capsid protein coding sequence is cloned. The gene is preferably expressed in a eukaryotic cell system. Insect cells are preferred host cells; however, yeast cells are also suitable as host cells if appropriate yeast expression vectors are used. Mammalian cells similarly transfected using appropriate mammalian expression vectors can also be used to produce assembled capsid

-11-

protein, however, cultured mammalian cells are less advantageous because they are more likely than non-mammalian cells to harbor occult viruses which might be infectious for mammals.

According to a preferred protocol, a baculovirus system is used. The gene to be cloned, substantially all of the coding sequence for bovine papillomavirus (BPV1) or human papillomavirus (HPV16) L1 capsid protein, or human papillomavirus HPV16 L1 and L2, is inserted into a baculovirus transfer vector containing flanking baculovirus sequences to form a gene construct, and the recombinant DNA is co-transfected with wild type baculovirus DNA into Sf-9 insect cells as described in Example 1, to generate recombinant virus which, on infection, can express the inserted gene at high levels. The actual production of protein is made by infecting fresh insect cells with the recombinant baculovirus; accordingly, the L1 capsid protein and the L1 and L2 capsid proteins are expressed in insect cells that have been infected with recombinant baculovirus as described in Example 2.

In the procedure of Example 1, the complete L1 gene of BPV1 was amplified by polymerase chain reaction (PCR; Saiki, R., et al., 1987) and cloned into AcMNPV (*Autographa californica* nuclear polyhedrosis virus) based baculovirus vector (Summers, M. et al., 1987). The L1 open reading frame was put under the control of the baculovirus polyhedrin promoter. After co-transfection of the L1 clone with the wild type (wt) baculovirus DNA into Sf-9 insect cells (ATCC Accession No. CRL 1711) and plaque purification of recombinant clones, high titer recombinant virus was generated. Extracts from cells infected with wt AcMNPV or BPV1 L1 recombinant viruses (AcBPV-L1) (Example 2) were analyzed by polyacrylamide gel electrophoresis. After Coomassie blue staining, a unique protein of the predicted size, 55 kilodaltons, was detected in extracts from the cultures infected with the AcBPV1-L1 virus. The identity of this protein as BPV L1 was verified by immunoblotting, using a BPV L1 specific monoclonal antibody (Nakai, Y., et al., 1986). Thus, the expression of BPV L1 by means of recombinant virus were demonstrated by SDS-PAGE analysis of lysates from infected insect cells.

To test the hypothesis that papillomavirus L1 has the ability to self-assemble into virus-like particles when overexpressed in heterologous cells, electron micrographs of thin sections from AcBPV-L1 infected cells were examined for the presence of papillomavirus-like structures. Cells infected with the BPV recombinant virus contained many circular structures of approximately 50 nm which were preferentially localized in the nucleus; these structures were absent from wild type baculovirus infected cells. These results suggested that self assembly of L1 into virus-like particles had occurred, since in vivo papillomavirus

-12-

virion assembly takes place in the nucleus and the diameter of the virions has been reported as 55 nm.

Following expression of the conformed capsid protein in the host cell, virus particles are purified from lysates of infected cells as described in Example 4. To obtain further evidence that the L1 protein had self-assembled, virus-like particles were isolated from the infected insect cells by means of gradient centrifugation. We demonstrated the conformation of purified recombinant BPV L1 and HPV16 L1 capsid proteins by electron microscopy, compared with authentic BPV virions.

High molecular mass structures were separated from lysates of L1 recombinant or wild type infected cells by centrifugation through a 40% sucrose cushion and the pelleted material was subjected to CsCl density gradient centrifugation. Fractions were collected and tested for reactivity to the BPV L1 specific monoclonal antibody by immunoblotting.

L1 positive fractions from the gradient were adsorbed onto carbon film grids, stained with 1% uranyl acetate and examined by transmission electron microscopy. In electron microscopy, the positive fractions contained numerous circular structures that exhibited a regular array of capsomers. Consistent with previous reports of the density of empty BPV virions (Larsen, P., et al., 1987), the density of the CsCl fraction containing the peak of the virus-like particles was approximately 1.30 gm/ml. Most were approximately 50 nm in diameter, although smaller circles and partially assembled structures were also seen. In electron microscopy, the larger particles were very similar in size and subunit structure to infectious BPV virions that had been stained and photographed concurrently. These particles were not observed in preparations from mock infected or wt AcMNPV infected cells. These results indicate that BPV L1 has the intrinsic capacity to assemble into virus-like particles in the absence of L2 or other papillomavirus proteins. In addition, specific factors limited to differentiating epithelia or mammalian cells are not required for papillomavirus capsid assembly.

To determine if the ability to self-assemble in insect cells is a general feature of papillomavirus L1, we also expressed the L1 of HPV16, the HPV type most often detected in human genital cancers, via an analogous recombinant baculovirus. A protein of the expected 58 kd size was expressed at high levels in the insect cells infected with the HPV16-L1 recombinant virus, as demonstrated by SDS-PAGE. This protein reacted strongly with an HPV16 L1 monoclonal antibody upon immunoblotting. The monoclonal antibody also lightly stained five other bands ranging in apparent molecular weight from approximately 28 kd to approximately 48 kd. The antibody also reacted weakly with BPV L1, thus this

-13-

antibody lightly stained the 55 kd protein of BPV L1 on the same immunoblot. After CsCl gradient purification, immunoreactive fractions were examined by electron microscopy and found to contain 50 nm papillomavirus-like particles upon electron microscopy. Although somewhat fewer completely assembled particles were seen in the human system in comparison to the BPV L1 preparations, possibly due to the lower levels of expression or greater extent of HPV16 L1 degradation seen in SDS-PAGE, the results conclusively indicate that the L1 of the HPV16 and presumably the L1 proteins of other types, have the intrinsic capacity to assemble into virion-type structures. Preparations of recombinant papillomavirus capsid particles for Rhesus monkey PV have also been carried out as described in the Examples.

Recombinant Conformed Capsid Proteins as Immunogens

Subunit vaccines, based on self-assembled major capsid proteins synthesized in heterologous cells, have been proved effective in preventing infections by several pathogenic viruses, including human hepatitis B (Stevens, C., et al., 1987).

Studies demonstrating that infectious or formalin inactivated BPV is effective as a vaccine, while BPV transformed cells are ineffective, suggest that viral capsid proteins, rather than early gene products, elicit the immune response. Other data in the scientific literature indicates that L1 protein extracted from bacteria was partially successful in eliciting an immune response despite the low titers of neutralizing antibodies. Accordingly, the BPV L1 that was expressed and assembled into virus-like particles in insect cells was studied for its ability to induce neutralizing antisera in rabbits. Two types of preparations were tested: whole cell extracts of L1 recombinant or wild type infected Sf-9 cells and partially purified particles isolated by differential centrifugation and ammonium sulfate precipitation. Following a primary inoculation, the rabbits received two biweekly booster inoculations.

The rabbit sera were tested for the ability to inhibit BPV infection of mouse C127 cells, as measured by a reduction in the number of foci induced by a standard amount of BPV virus. A representative assay was conducted in which the titers of neutralizing antisera induced in animals inoculated with recombinant BPV L1 was compared to antisera against intact and denatured BPV virions. The immune sera generated by inoculation with baculovirus derived L1 were able to reduce the infectivity of the BPV virus by 50% at a dilution of at least 1:11,000 (a titer of 11,000; Table 1), whereas the preimmune sera from the same rabbits did not inhibit focal transformation at a dilution of 1:20, the lowest dilution tested. Both the crude preparations and partially purified particles were effective in

-14-

inducing high titer neutralizing antisera, with 290,000 being the highest titer measured. This was the same as the neutralizing titer of the positive control antiserum raised against infectious BPV virions. In comparison, the highest titer generated in a previous study using bacterially derived L1 was 36 (Pilancinski, W., et al., 1984). The serum from the rabbit inoculated with the extract from the wild type baculovirus infected cells was unable to inhibit infectivity at a dilution of 1:20, indicating that the neutralizing activity was L1 specific. Disruption of the partially purified L1 particles, by boiling in 1% SDS, abolished the ability of the preparation to induce neutralizing antibodies (Table 1). The demonstration that L1 can self-assemble into virion-like particles that elicit neutralizing antisera titers at least three orders of magnitude higher than previous in vitro-produced antigens suggests the recombinant L1 capsid proteins has the potential to induce effective long term protection against naturally transmitted papillomavirus. In view of these results, it appears that the L1 particles assembled in insect cells mimic infectious virus in the presentation of conformationally dependent immunodominant epitopes. These results also establish that L2 is not required for the generation of high titer neutralizing antibodies. The reported weak neutralizing immunogenicity of bacterially derived L1 may occur because it does not assume an appropriate conformation or has not assembled into virion like structures. Also, multiple electrophoretic variants of L1 have been detected in virions (Larsen, P., et al., 1987). Some of these modified species, which are probably absent in the bacterially derived L1, may facilitate the generation of neutralizing antibodies.

The ability of recombinant L1 (or L2) papillomavirus capsid proteins such as those disclosed herein to induce high titer neutralizing antiserum makes them suitable for use as vaccines for prophylaxis against communicable papillomatosis. Examples of populations at risk that could benefit from immunization are bovine herds, which are susceptible to papilloma warts; all humans for non-genital types of HPV infection; and sexually active humans for genital HPV types of infection.

Therapeutic vaccination can be useful for productive papillomavirus lesions, which usually express L1 (and L2) capsid proteins. Such lesions are most likely to occur in benign infections, such as warts or laryngeal papillomatosis. Laryngeal papillomatosis in newborns is usually contracted by the infant during passage through the birth canal where infectious papillomavirus is present in vaginal secretions. Therapeutic vaccination of infected pregnant women against the papillomavirus can induce neutralizing IgG antibody capable of passing through the placental barrier and into the circulation of the fetus to provide prophylactic passive immunity in the infant against this type of papillomavirus infection. Additional

-15-

infant-protecting mechanisms are provided by maternal IgA which is secreted into the vaginal fluid and into breast milk. Jarrett (1991) demonstrates some therapeutic efficacy for L2 in treating BPV-induced warts. Malignant tumors typically do not express L1 or L2, and the efficacy of vaccination with recombinant L1 or L2 in conditions such as cervical cancer, is uncertain.

Protective immunity against both benign and malignant papillomavirus disease can be induced by administering an effective amount of recombinant L1 capsid protein to an individual at risk for papillomavirus infection. A vaccine comprising the capsid protein can be directly administered, either parenterally or locally, according to conventional immunization protocols. In an alternative embodiment, the conformational coding sequence of L1 can be cloned into a transfer vector, for example, a semliki forest virus vector (which produces a mild transient infection), the recombinant virus introduced into the cells or tissues of the recipient where the immunizing capsid protein is then expressed. Vaccinia virus can also be used as a vehicle for the gene.

Recombinant Conformed Capsid Proteins as Serological Screening Agents

Published serologic studies of human immune response to papillomavirus virion proteins have principally utilized bacterially derived L1 and L2 capsid proteins, and the results have not correlated well with other measures of HPV infection (Jenison, S., et al., 1990). BPV papillomavirus immunity studies described above indicate that papillomavirus virion proteins extracted from bacteria do not present the conformationally dependent epitopes that appear to be type-specific and recognized by most neutralizing antibodies. Compared with such assays that primarily recognize linear epitopes, a serological test using self-assembled L1 particles is likely to be a more accurate measure of the extent of anti-HPV virion immunity in the human population. The recombinant L1 capsid proteins disclosed herein, presenting conformational epitopes, can therefore be used as highly specific diagnostic reagents to detect immunity conferring neutralizing antibody to papilloma virus in binding assays of several types. The procedures can be carried out generally as either solid phase or solution assays that provide a means to detect antibodies in bodily fluids that specifically bind to the capsid protein in antigen-antibody pairs. Examples of procedures known to those skilled in the art for evaluating circulating antibodies are solution phase assays, such as double-antibody radioimmunoassays or enzyme immunoassays, or solid phase assays such as strip radioimmunoassay based on Western blotting or an enzyme-linked immunoabsorbent assay (ELISA) as disclosed in U.S. Patent No. 4,520,113 to Gallo et al.,

-16-

or immunochromatographic assays as disclosed in U.S. Patent No. 5,039,607 to Skold et al.

A preferred ELISA method for the detection of antibodies is that disclosed in Harlow, E., and Lane, D. in Antibodies: A Laboratory Manual Cold Spring Harbor, NY, 1988, pp. 563-578.

5 The recombinant L1 or L1/L2 capsid proteins disclosed herein can also be used to measure cellular immunity to papillomavirus by means of in vivo or in vitro assays, for example, antigen-induced T-cell proliferative responses as described by Bradley, L., 1980, and particularly cellular responses to viral antigens, as described in U.S. Patent No. 5,081,029 to Starling. Cellular immunity to papillomavirus can also be determined by the
10 classical in vivo delayed hypersensitivity skin test as described by Stites, D., 1980; or in a preferred method, according to Höpfel, R., et al., 1991, by the intradermal injection of recombinant HPV L1 fusion proteins.

 The capsid proteins of the invention can also be used as immunogens to raise polyclonal or monoclonal antibodies, according to methods well known in the art. These
15 papillomavirus-specific antibodies, particularly in combination with labelled second antibodies, specific for a class or species of antibodies, can be used diagnostically according to various conventional assay procedures, such as immunohistochemistry, to detect the presence of capsid proteins in samples of body tissue or bodily fluids.

 The genetic manipulations described below are disclosed in terms of their general
20 application to the preparation of elements of the genetic regulatory unit of the invention. Occasionally, the procedure may not be applicable as described to each recombinant molecule included within the disclosed scope. The situations for which this occurs will be readily recognized by those skilled in the art. In all such cases, either the operations can be successfully performed by conventional modifications known to those skilled in the art,
25 e.g. by choice of an appropriate alternative restriction enzyme, by changing to alternative conventional reagents, or by routine modification of reaction conditions. Alternatively, other procedures disclosed herein or otherwise conventional will be applicable to the preparation of the corresponding recombinant molecules of the invention. In all preparative methods, all starting materials are known or readily preparable from known starting materials. In the
30 following examples, all temperatures are set forth in degrees Celsius; unless otherwise indicated, all parts and percentages are by weight.

 Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the invention to its fullest extent. The following preferred

-17-

embodiments are therefore to be construed as merely illustrative and not limiting the remainder of the disclosure in any way whatsoever.

EXAMPLE 1

5 Full length L1, or L1 and L2 open reading frames (ORF) were amplified by PCR using the cloned prototypes of BPV1 DNA (Chen, E., et al., 1982), GenBank Accession No. X02346 or HPV16 DNA (Seedorf, K., et al., 1985), GenBank Accession No. K02718; or wild type HPV16 DNA SEQ ID NO:2) as templates. Unique restriction sites were incorporated into the oligonucleotide primers (underlined).

10 BPV1-L1 primer sequence SEQ ID NO:3):
 5'-CCGCTGAATTCAATATGGCGTTGTGGCAACAAGGCCAGAAGCTGTAT-3' (sense)
 and SEQ ID NO:4):
 5'-GCGGTGGTACCGTGCAGTTGACTTACCTTCTGTTTTACATTACAGA-3'
 (antisense);

15 HPV16-L1 primer sequence SEQ ID NO:5):
 5'-CCGCTAGATCTAATATGTCTCTTTGGCTGCCTAGTGAGGCC-3' (sense); and
 SEQ ID NO:6):
 5'-GCGGTAGATCTACACTAATTCAACATACATACTTACAGC-3'(antisense).

20 L1 coding sequences begin at the 1st methionine codon (bold) for BPV1 and the 2nd methionine for HPV16. BPV1-L1 was cloned as a 5'-EcoRI to 3'-KpnI fragment and HPV16-L1 as a 5'-BglII to 3'-BglII fragment into the multiple cloning site downstream of the polyhedrin promoter of the AcMNPV based baculovirus transfer vector pEV mod (Wang, X., et al. 1991) and verified by sequencing through the AcMNPV/L1 junction. A quantity of 2 µg of CsCl-purified recombinant plasmid was cotransfected with 1 µg wild type

25 AcMNPV DNA (Invitrogen, San Diego, California) into Sf-9 cells (ATCC) using lipofectin (Gibco/BRL, Gaithersburg, Maryland) (Hartig, P., et al., 1991) and the recombinant baculoviruses plaque-purified as described (Summers, M., et al., 1987).

EXAMPLE 2

30 Expression of L1 Proteins or L1/L2 proteins in Insect Cells

Sf-9 cells were either mock infected (mock) or infected at a multiplicity of infection of 10 with either wt AcMNPV (wt) or AcBPV-L1 (B-L1), AcHPV16-L1 (16-L1), or AcHPV16-L1 (16-L1) and AcHPV16-L2 (16-L2) recombinant virus. After 72 hours, cells were lysed by boiling in Laemmli buffer and the lysates subjected to SDS-PAGE in 10%

-18-

gels. Proteins were either stained with 0.25% Coomassie blue or immunoblotted and probed with BPV L1 mAb AU-1 (Nakai, Y., et al., 1986), or HPV16L1 mAb CAMVIR-1 (McLean, C., et al., 1990) and ^{125}I -labeled Fab anti-mouse IgG (Amersham). P designates polyhedrin protein. The anti BPV L1 mAb recognized the expected 55 kd protein. The anti-HPV16L1 mAb strongly stained the expected 58 kd protein, as well as lightly staining five lower molecular weight bands, as discussed above. As also discussed above, this anti-HPV16L1 lightly cross-reacted with the BPV L1 protein.

EXAMPLE 3

Production of antisera

Rabbits were immunized by subcutaneous injection either with whole cell Sf-9 lysates (3×10^7 cells) prepared by one freeze/thaw cycle and 20x dounce homogenization (rabbit #1,2, and 8) or with 200 μg of L1 protein partially purified by differential centrifugation and 35% ammonium sulfate precipitation (#3,4,6, and 7), in complete Freund's adjuvant, and then boosted twice at two week intervals, using the same preparations in incomplete Freund's adjuvant.

EXAMPLE 4

Purification of Particles and Transmission Electron Microscopic (EMK) Analysis

500 ml of Sf-9 cells ($2 \times 10^6/\text{ml}$) were infected with AcBPV-L1 or AcHPV16-L1 or AcHPV16-L1/L2 (16-L1/L2) recombinant baculoviruses. After 72 hr, the harvested cells were sonicated in PBS for 60 sec. After low speed clarification, the lysates were subjected to centrifugation at 110,000g for 2.5 hr through a 40% (wt/vol) sucrose/PBS cushion (SW-28). The resuspended pellets were centrifuged to equilibrium at 141,000g for 20 hr at room temperature in a 10-40% (wt/wt) CsCl /PBS gradient. Fractions were harvested from the bottom and analyzed by SDS-PAGE. Immunoreactive fractions were dialyzed against PBS, concentrated by Centricon 30 (Millipore) ultrafiltration, and (for HPV16-L1) pelleted by centrifugation for 10 min at 30 psi in a A-100 rotor in an airfuge (Beckman). BPV1 virions (Fig. 2B) were purified from a bovine wart (generously provided by A.B. Jensen) as described (Cowser, L., et al., 1987). Purified particles were adsorbed to carbon coated TEM grids, stained with 1% uranyl acetate and examined with a Philips electron microscope EM 400T at 36,000x magnification. Results were obtained by electron microscopy, and are discussed above.

-19-
EXAMPLE 5

BPV1 neutralization assay

Serial dilutions of sera obtained 3 wk after the second boost were incubated with approximately 500 focus forming units of BPV1 virus for 30 min, the virus absorbed to C127
5 cells for 1 hr and the cells cultured for 3 weeks (Dvoretzky, I., et al., 1980). The foci were stained with 0.5% methylene blue/0.25% carbol fuchsin/methanol. The results were obtained by evaluating the number of foci; these results are discussed below. Anti-AcBPV-L1 was obtained from rabbit #1 and anti-wt AcMNPV from rabbit #8 (Table 1). Preimmune sera at 1:400 dilution was used as a standard. Anti-AcBPV-L1 at either
10 1:400 or 1:600 dilution substantially eliminated foci, whereas anti-wt AcMNPV at either 1:400 or 1:600 dilution appeared to produce an increase in the number of foci. The normal rabbit serum negative control designated "nrs" at 1:00 dilution was used as a standard for the anti-BPV-1 virion, which appeared to substantially eliminate foci at either 1:400 or 1:600 dilution. The anti-BPV-1 virion was raised against native BPV virions in a previous study
15 (Nakai, Y., et al., 1986). Finally, Dako is the commercially available (Dako Corp., Santa Barbara, California) rabbit antiserum raised against denatured BPV virions. This serum produced a large number of foci, apparently greater than a no Ab control. As a negative control, a no virus test produced substantially no foci.

20

EXAMPLE 6

Serum Neutralizing Titer against BPV1

Assays were carried out as in Example 5. Rabbits #1, 2, and 8 were inoculated with crude whole cell Sf-9 lysates, and rabbits # 3,4,6, and 7 with partially purified L1 protein (Table 1). Rabbits #6 and 7 were immunized with L1 protein preparations that had been
25 denatured by boiling in 1% SDS. At least two bleeds, taken 3-6 weeks after the second boost, were tested for each rabbit and found to have the same titer. The titer of the preimmune sera from each of the rabbits was less than 20, the lowest dilution tested.

-20-
TABLE 1

Antigen	rabbit	serum neutralization titer against BPV1*
AcBPV-L1	1	11,000
" "	2	97,000
" "	3	290,000
" "	4	97,000
BPV1-virion†	5	290,000
AcBPV-L1/SDS	6	<2
" "	7	<2
wt AcMNPV	8	<20

*reciprocal of dilution that caused 50% focus reduction

†provided by A.B. Jenson (Nakai, Y., et al., 1986).

-21-
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-23-

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: The Government of the United States, as represented by the Secretary of Health and Human Services

(ii) TITLE OF INVENTION: SELF- ASSEMBLING RECOMBINANT PAPILLOMAVIRUS CAPSID PROTEINS

(iii) NUMBER OF SEQUENCES: 6

(iv) CORRESPONDENCE ADDRESS:

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(v) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
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- (D) SOFTWARE: PatentIn Release #1.0, Version #1.25

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(ix) TELECOMMUNICATION INFORMATION:

- (A) TELEPHONE: 714-760-0404
- (B) TELEFAX: 714-760-9502

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1517 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Human papillomavirus
- (B) STRAIN: HPV16

-24-

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..1518

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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CCA	GTA	TCT	AAG	GTT	GTA	AGC	ACG	GAT	GAA	TAT	GTT	GCA	CGC	ACA	AAC	96
Pro	Val	Ser	Lys	Val	Val	Ser	Thr	Asp	Glu	Tyr	Val	Ala	Arg	Thr	Asn	
			20					25					30			
ATA	TAT	TAT	CAT	GCA	GGA	ACA	TCC	AGA	CTA	CTT	GCA	GTT	GGA	CAT	CCC	144
Ile	Tyr	Tyr	His	Ala	Gly	Thr	Ser	Arg	Leu	Leu	Ala	Val	Gly	His	Pro	
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TAT	TTT	CCT	ATT	AAA	AAA	CCT	AAC	AAT	AAC	AAA	ATA	TTA	GTT	CCT	AAA	192
Tyr	Phe	Pro	Ile	Lys	Lys	Pro	Asn	Asn	Asn	Lys	Ile	Leu	Val	Pro	Lys	
	50					55					60					
GTA	TCA	GGA	TTA	CAA	TAC	AGG	GTA	TTT	AGA	ATA	CAT	TTA	CCT	GAC	CCC	240
Val	Ser	Gly	Leu	Gln	Tyr	Arg	Val	Phe	Arg	Ile	His	Leu	Pro	Asp	Pro	
65				70				75						80		
AAT	AAG	TTT	GGT	TTT	CCT	GAC	ACC	TCA	TTT	TAT	AAT	CCA	GAT	ACA	CAG	288
Asn	Lys	Phe	Gly	Phe	Pro	Asp	Thr	Ser	Phe	Tyr	Asn	Pro	Asp	Thr	Gln	
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CGG	CTG	GTT	TGG	GCC	TGT	GTA	GGT	GTT	GAG	GTA	GGT	CGT	GGT	CAG	CCA	336
Arg	Leu	Val	Trp	Ala	Cys	Val	Gly	Val	Glu	Val	Gly	Arg	Gly	Gln	Pro	
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TTA	GGT	GTG	GGC	ATT	AGT	GGC	CAT	CCT	TTA	TTA	AAT	AAA	TTG	GAT	GAC	384
Leu	Gly	Val	Gly	Ile	Ser	Gly	His	Pro	Leu	Leu	Asn	Lys	Leu	Asp	Asp	
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ACA	GTT	ATT	CAG	GAT	GGT	GAT	ATG	GTT	CAT	ACT	GGC	TTT	GGT	GCT	ATG	624
Thr	Val	Ile	Gln	Asp	Gly	Asp	Met	Val	His	Thr	Gly	Phe	Gly	Ala	Met	
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-25-

GAC Asp	TTT Phe	ACT Thr	ACA Thr	TTA Leu	CAG Gln	GCT Ala	AAC Asn	AAA Lys	AGT Ser	GAA Glu	GTT Val	CCA Pro	CTG Leu	GAT Asp	ATT Ile	672
210						215				220						
TGT Cys	ACA Thr	TCT Ser	ATT Ile	TGC Cys	AAA Lys	TAT Tyr	CCA Pro	GAT Asp	TAT Tyr	ATT Ile	AAA Lys	ATG Met	GTG Val	TCA Ser	GAA Glu	720
225					230					235					240	
CCA Pro	TAT Tyr	GGC Gly	GAC Asp	AGC Ser	TTA Leu	TTT Phe	TTT Phe	TAT Tyr	TTA Leu	CGA Arg	AGG Arg	GAA Glu	CAA Gln	ATG Met	TTT Phe	768
			245						250					255		
GTT Val	AGA Arg	CAT His	TTA Leu	TTT Phe	AAT Asn	AGG Arg	GCT Ala	GGT Gly	ACT Thr	GTT Val	GGT Gly	GAA Glu	AAT Asn	GTA Val	CCA Pro	816
			260					265					270			
GAC Asp	GAT Asp	TTA Leu	TAC Tyr	ATT Ile	AAA Lys	GGC Gly	TCT Ser	GGG Gly	TCT Ser	ACT Thr	GCA Ala	AAT Asn	TTA Leu	GCC Ala	AGT Ser	864
		275					280					285				
TCA Ser	AAT Asn	TAT Tyr	TTT Phe	CCT Pro	ACA Thr	CCT Pro	AGT Ser	GGT Gly	TCT Ser	ATG Met	GTT Val	ACC Thr	TCT Ser	GAT Asp	GCC Ala	912
		290				295					300					
CAA Gln	ATA Ile	TTC Phe	AAT Asn	AAA Lys	CCT Pro	TAT Tyr	TGG Trp	TTA Leu	CAA Gln	CGA Arg	GCA Ala	CAG Gln	GGC Gly	CAC His	AAT Asn	960
305					310					315					320	
AAT Asn	GGC Gly	ATT Ile	TGT Cys	TGG Trp	GGT Gly	AAC Asn	CAA Gln	CTA Leu	TTT Phe	GTT Val	ACT Thr	GTT Val	GTT Val	GAT Asp	ACT Thr	1008
				325					330					335		
ACA Thr	CGC Arg	AGT Ser	ACA Thr	AAT Asn	ATG Met	TCA Ser	TTA Leu	TGT Cys	GCT Ala	GCC Ala	ATA Ile	TCT Ser	ACT Thr	TCA Ser	GAA Glu	1056
			340					345					350			
ACT Thr	ACA Thr	TAT Tyr	AAA Lys	AAT Asn	ACT Thr	AAC Asn	TTT Phe	AAG Lys	GAG Glu	TAC Tyr	CTA Leu	CGA Arg	CAT His	GGG Gly	GAG Glu	1104
		355					360					365				
GAA Glu	TAT Tyr	GAT Asp	TTA Leu	CAG Gln	TTT Phe	ATT Ile	TTT Phe	CAA Gln	CTG Leu	TGC Cys	AAA Lys	ATA Ile	ACC Thr	TTA Leu	ACT Thr	1152
		370				375					380					
GCA Ala	GAC Asp	GTT Val	ATG Met	ACA Thr	TAC Tyr	ATA Ile	CAT His	TCT Ser	ATG Met	AAT Asn	TCC Ser	ACT Thr	ATT Ile	TTG Leu	GAG Glu	1200
					390					395					400	
GAC Asp	TGG Trp	AAT Asn	TTT Phe	GGT Gly	CTA Leu	CAA Gln	CCT Pro	CCC Pro	CCA Pro	GGA Gly	GGC Gly	ACA Thr	CTA Leu	GAA Glu	GAT Asp	1248
				405					410					415		
ACT Thr	TAT Tyr	AGG Arg	TTT Phe	GTA Val	ACA Thr	TCC Ser	CAG Gln	GCA Ala	ATT Ile	GCT Ala	TGT Cys	CAA Gln	AAA Lys	CAT His	ACA Thr	1296
			420					425					430			
CCT Pro	CCA Pro	GCA Ala	CCT Pro	AAA Lys	GAA Glu	GAT Asp	CCC Pro	CTT Leu	AAA Lys	AAA Lys	TAC Tyr	ACT Thr	TTT Phe	TGG Trp	GAA Glu	1344
		435					440					445				

-26-

GTA AAT TTA AAG GAA AAG TTT TCT GCA GAC CTA GAT CAG TTT CCT TTA	1392
Val Asn Leu Lys Glu Lys Phe Ser Ala Asp Leu Asp Gln Phe Pro Leu	
450 455 460	
GGA CGC AAA TTT TTA CTA CAA GCA GGA TTG AAG GCC AAA CCA AAA TTT	1440
Gly Arg Lys Phe Leu Leu Gln Ala Gly Leu Lys Ala Lys Pro Lys Phe	
465 470 475 480	
ACA TTA GGA AAA CGA AAA GCT ACA CCC ACC ACC TCA TCT ACC TCT ACA	1488
Thr Leu Gly Lys Arg Lys Ala Thr Pro Thr Thr Ser Ser Thr Ser Thr	
485 490 495	
ACT GCT AAA CGC AAA AAA CGT AAG CTG TA	1518
Thr Ala Lys Arg Lys Lys Arg Lys Leu	
500 505	

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1518 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..1518

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

ATG TCT CTT TGG CTG CCT AGT GAG GCC ACT GTC TAC TTG CCT CCT GTC	48
Met Ser Leu Trp Leu Pro Ser Glu Ala Thr Val Tyr Leu Pro Pro Val	
1 5 10 15	
CCA GTA TCT AAG GTT GTA AGC ACG GAT GAA TAT GTT GCA CGC ACA AAC	96
Pro Val Ser Lys Val Val Ser Thr Asp Glu Tyr Val Ala Arg Thr Asn	
20 25 30	
ATA TAT TAT CAT GCA GGA ACA TCC AGA CTA CTT GCA GTT GGA CAT CCC	144
Ile Tyr Tyr His Ala Gly Thr Ser Arg Leu Leu Ala Val Gly His Pro	
35 40 45	
TAT TTT CCT ATT AAA AAA CCT AAC AAT AAC AAA ATA TTA GTT CCT AAA	192
Tyr Phe Pro Ile Lys Lys Pro Asn Asn Asn Lys Ile Leu Val Pro Lys	
50 55 60	
GTA TCA GGA TTA CAA TAC AGG GTA TTT AGA ATA CAT TTA CCT GAC CCC	240
Val Ser Gly Leu Gln Tyr Arg Val Phe Arg Ile His Leu Pro Asp Pro	
65 70 75 80	

-27-

AAT	AAG	TTT	GGT	TTT	CCT	GAC	ACC	TCA	TTT	TAT	AAT	CCA	GAT	ACA	CAG	288
Asn	Lys	Phe	Gly	Phe	Pro	Asp	Thr	Ser	Phe	Tyr	Asn	Pro	Asp	Thr	Gln	
				85					90					95		
CGG	CTG	GTT	TGG	GCC	TGT	GTA	GGT	GTT	GAG	GTA	GGT	CGT	GGT	CAG	CCA	336
Arg	Leu	Val	Trp	Ala	Cys	Val	Gly	Val	Glu	Val	Gly	Arg	Gly	Gln	Pro	
			100					105					110			
TTA	GGT	GTG	GGC	ATT	AGT	GGC	CAT	CCT	TTA	TTA	AAT	AAA	TTG	GAT	GAC	384
Leu	Gly	Val	Gly	Ile	Ser	Gly	His	Pro	Leu	Leu	Asn	Lys	Leu	Asp	Asp	
		115					120					125				
ACA	GAA	AAT	GCT	AGT	GCT	TAT	GCA	GCA	AAT	GCA	GGT	GTG	GAT	AAT	AGA	432
Thr	Glu	Asn	Ala	Ser	Ala	Tyr	Ala	Ala	Asn	Ala	Gly	Val	Asp	Asn	Arg	
	130					135					140					
GAA	TGT	ATA	TCT	ATG	GAT	TAC	AAA	CAA	ACA	CAA	TTG	TGT	TTA	ATT	GGT	480
Glu	Cys	Ile	Ser	Met	Asp	Tyr	Lys	Gln	Thr	Gln	Leu	Cys	Leu	Ile	Gly	
145					150					155					160	
TGC	AAA	CCA	CCT	ATA	GGG	GAA	CAC	TGG	GGC	AAA	GGA	TCC	CCA	TGT	ACC	528
Cys	Lys	Pro	Pro	Ile	Gly	Glu	His	Trp	Gly	Lys	Gly	Ser	Pro	Cys	Thr	
				165					170					175		
AAT	GTT	GCA	GTA	AAT	CCA	GGT	GAT	TGT	CCA	CCA	TTA	GAG	TTA	ATA	AAC	576
Asn	Val	Ala	Val	Asn	Pro	Gly	Asp	Cys	Pro	Pro	Leu	Glu	Leu	Ile	Asn	
			180					185					190			
ACA	GTT	ATT	CAG	GAT	GGT	GAT	ATG	GTT	GAT	ACT	GGC	TTT	GGT	GCT	ATG	624
Thr	Val	Ile	Gln	Asp	Gly	Asp	Met	Val	Asp	Thr	Gly	Phe	Gly	Ala	Met	
		195					200					205				
GAC	TTT	ACT	ACA	TTA	CAG	GCT	AAC	AAA	AGT	GAA	GTT	CCA	CTG	GAT	ATT	672
Asp	Phe	Thr	Thr	Leu	Gln	Ala	Asn	Lys	Ser	Glu	Val	Pro	Leu	Asp	Ile	
	210					215						220				
TGT	ACA	TCT	ATT	TGC	AAA	TAT	CCA	GAT	TAT	ATT	AAA	ATG	GTG	TCA	GAA	720
Cys	Thr	Ser	Ile	Cys	Lys	Tyr	Pro	Asp	Tyr	Ile	Lys	Met	Val	Ser	Glu	
225					230					235					240	
CCA	TAT	GGC	GAC	AGC	TTA	TTT	TTT	TAT	TTA	CGA	AGG	GAA	CAA	ATG	TTT	768
Pro	Tyr	Gly	Asp	Ser	Leu	Phe	Phe	Tyr	Leu	Arg	Arg	Glu	Gln	Met	Phe	
				245					250					255		
GTT	AGA	CAT	TTA	TTT	AAT	AGG	GCT	GGT	ACT	GTT	GGT	GAA	AAT	GTA	CCA	816
Val	Arg	His	Leu	Phe	Asn	Arg	Ala	Gly	Thr	Val	Gly	Glu	Asn	Val	Pro	
			260					265					270			
GAC	GAT	TTA	TAC	ATT	AAA	GGC	TCT	GGG	TCT	ACT	GCA	AAT	TTA	GCC	AGT	864
Asp	Asp	Leu	Tyr	Ile	Lys	Gly	Ser	Gly	Ser	Thr	Ala	Asn	Leu	Ala	Ser	
		275					280					285				
TCA	AAT	TAT	TTT	CCT	ACA	CCT	AGT	GGT	TCT	ATG	GTT	ACC	TCT	GAT	GCC	912
Ser	Asn	Tyr	Phe	Pro	Thr	Pro	Ser	Gly	Ser	Met	Val	Thr	Ser	Asp	Ala	
	290					295					300					
CAA	ATA	TTC	AAT	AAA	CCT	TAT	TGG	TTA	CAA	CGA	GCA	CAG	GGC	CAC	AAT	960
Gln	Ile	Phe	Asn	Lys	Pro	Tyr	Trp	Leu	Gln	Arg	Ala	Gln	Gly	His	Asn	
305					310					315					320	

-28-

AAT GGC ATT TGT TGG GGT AAC CAA CTA TTT GTT ACT GTT GTT GAT ACT	1008
Asn Gly Ile Cys Trp Gly Asn Gln Leu Phe Val Thr Val Val Asp Thr	
325 330 335	
ACA CGC AGT ACA AAT ATG TCA TTA TGT GCT GCC ATA TCT ACT TCA GAA	1056
Thr Arg Ser Thr Asn Met Ser Leu Cys Ala Ala Ile Ser Thr Ser Glu	
340 345 350	
ACT ACA TAT AAA AAT ACT AAC TTT AAG GAG TAC CTA CGA CAT GGG GAG	1104
Thr Thr Tyr Lys Asn Thr Asn Phe Lys Glu Tyr Leu Arg His Gly Glu	
355 360 365	
GAA TAT GAT TTA CAG TTT ATT TTT CAA CTG TGC AAA ATA ACC TTA ACT	1152
Glu Tyr Asp Leu Gln Phe Ile Phe Gln Leu Cys Lys Ile Thr Leu Thr	
370 375 380	
GCA GAC GTT ATG ACA TAC ATA CAT TCT ATG AAT TCC ACT ATT TTG GAG	1200
Ala Asp Val Met Thr Tyr Ile His Ser Met Asn Ser Thr Ile Leu Glu	
385 390 395 400	
GAC TGG AAT TTT GGT CTA CAA CCT CCC CCA GGA GGC ACA CTA GAA GAT	1248
Asp Trp Asn Phe Gly Leu Gln Pro Pro Pro Gly Gly Thr Leu Glu Asp	
405 410 415	
ACT TAT AGG TTT GTA ACC CAG GCA ATT GCT TGT CAA AAA CAT ACA CCT	1296
Thr Tyr Arg Phe Val Thr Gln Ala Ile Ala Cys Gln Lys His Thr Pro	
420 425 430	
CCA GCA CCT AAA GAA GAT GAT CCC CTT AAA AAA TAC ACT TTT TGG GAA	1344
Pro Ala Pro Lys Glu Asp Asp Pro Leu Lys Lys Tyr Thr Phe Trp Glu	
435 440 445	
GTA AAT TTA AAG GAA AAG TTT TCT GCA GAC CTA GAT CAG TTT CCT TTA	1392
Val Asn Leu Lys Glu Lys Phe Ser Ala Asp Leu Asp Gln Phe Pro Leu	
450 455 460	
GGA CGC AAA TTT TTA CTA CAA GCA GGA TTG AAG GCC AAA CCA AAA TTT	1440
Gly Arg Lys Phe Leu Leu Gln Ala Gly Leu Lys Ala Lys Pro Lys Phe	
465 470 475 480	
ACA TTA GGA AAA CGA AAA GCT ACA CCC ACC ACC TCA TCT ACC TCT ACA	1488
Thr Leu Gly Lys Arg Lys Ala Thr Pro Thr Thr Ser Ser Thr Ser Thr	
485 490 495	
ACT GCT AAA CGC AAA AAA CGT AAG CTG TA	1517
Thr Ala Lys Arg Lys Lys Arg Lys Leu	
500 505	

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 47 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

-29-

- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Bovine papillomavirus
- (vii) IMMEDIATE SOURCE:
 - (B) CLONE: BPV1 N

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CCGCTGAATT CAATATGGCG TTGTGGCAAC AAGGCCAGAA GCTGTAT

47

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 47 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: YES
- (vii) IMMEDIATE SOURCE:
 - (B) CLONE: BPV1 Y

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GCGGTGGTAC CGTGCAATTG ACTTACCTTC TGTTTTACAT TTACAGA

47

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 41 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vii) IMMEDIATE SOURCE:
 - (B) CLONE: HPV16 N

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

- 30 -

CCGCTAGATC TAATATGTCT CTTTGGCTGC CTAGTGAGGC C

41

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 44 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)

- (iii) HYPOTHETICAL: NO

- (iv) ANTI-SENSE: YES

- (vii) IMMEDIATE SOURCE:
 - (B) CLONE: HPV16 Y

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GCGGTAGATC TACACTAATT CAACATACAT ACAATACTTA CAGC

44

-31-

WHAT IS CLAIMED IS:

1. A genetic construct, comprising a papillomavirus L1 conformational coding sequence, inserted into a baculovirus transfer vector, and operatively expressed by a promoter of that vector.

5 2. The genetic construct of Claim 1, wherein said papillomavirus L1 conformational coding sequence is isolated from a bovine, monkey, or human gene.

3. The genetic construct of Claim 2, wherein said papillomavirus L1 conformational coding sequence is isolated from a wild type HPV16 gene.

4. The genetic construct of Claim 3, wherein said papillomavirus L1 conformational coding sequence is SEQ ID NO:6.

10 5. The genetic construct of Claim 3, further comprising a papillomavirus L2 coding sequence.

6. A non-mammalian eukaryotic host cell transformed by the genetic construct of any one of Claims 1-5.

15 7. A method for producing a recombinant papillomavirus capsid protein, assembled into a capsomer structure or a portion thereof, comprising the steps of:

cloning a papillomavirus gene that codes for an L1 conformational capsid protein into a transfer vector wherein the open reading frame of said gene is under the control of the promoter of said vector;

20 transferring the recombinant vector into a host cell, wherein the cloned papillomavirus gene expresses said papillomavirus capsid protein; and

isolating capsomer structures, comprising said papillomavirus capsid protein, from said cell.

25 8. The method of Claim 7, wherein the cloned papillomavirus gene consists essentially of the conformational L1 coding sequence, and the expressed protein assembles into capsomer structures consisting essentially of L1 capsid protein.

9. The method of Claim 7, wherein the cloning step further comprises the cloning of a papillomavirus gene coding for L2 capsid protein, whereby said L1 and L2 proteins are coexpressed, and wherein the isolated capsomer structures comprise L1 and L2 capsid proteins;

30 provided that said transfer vector is not a vaccinia virus when said host cell is a mammalian cell.

10. The method of Claim 7 or 9 wherein the conformational L1 coding sequence is cloned from a bovine, monkey, or human papillomavirus.

-32-

11. The method of Claim 7 or 9, wherein the conformational L1 coding sequence is cloned from a wild type HPV16 papillomavirus.

12. The method of Claim 11, wherein said conformational L1 coding sequence is SEQ ID NO:6.

5 13. The method of Claim 7, wherein said host cell is an insect cell.

14. The method of Claim 7, wherein said vector is a baculovirus based transfer vector, and the papillomavirus gene is under the control of a promoter that is active in insect cells.

10 15. The method of Claim 7, wherein said recombinant baculovirus DNA is transfected into Sf-9 insect cells.

16. The method of Claim 15, wherein said recombinant baculovirus DNA is co-transfected with wild-type baculovirus DNA into Sf-9 insect cells.

17. The method of Claim 7, wherein said vector is a yeast transfer vector, and the recombinant vector is transfected into yeast cells.

15 18. A virus capsomer structure, or a portion thereof, consisting essentially of papillomavirus L1 capsid protein, produced by the method of Claim 7.

19. A virus capsomer structure, consisting essentially of papillomavirus L1 and L2 capsid proteins, produced by the method of Claim 9.

20 20. A virus capsomer structure according to Claim 18 or 19 wherein said papillomavirus L1 capsid protein is the expression product of an HPV16 L1 DNA cloned from a wild type virus.

21. A virus capsid or a capsomer structure, or a portion thereof, consisting essentially of papillomavirus L1 capsid protein.

25 22. A virus structure according to Claim 21, consisting essentially of wild type HPV16 papillomavirus L1 capsid protein.

23. A virus structure according to any one of Claims 18, 19, 21, or 22, wherein said capsid protein includes an immunogenic conformational epitope capable of inducing neutralizing antibodies against native papillomavirus.

30 24. A virus structure according to Claim 23, wherein said papillomavirus L1 capsid protein is selected from the group consisting of bovine, monkey, or human papillomavirus L1 proteins.

25. A virus structure according to Claim 24, wherein said papillomavirus L1 capsid protein is the expression product of a wild type HPV16 L1 gene.

-33-

26. A virus structure according to Claim 25, wherein said HPV16 L1 gene comprises the sequence of SEQ ID NO:6.

27. A unit dose of a vaccine, comprising a peptide having conformational epitopes of a papillomavirus L1 capsid protein, or L1 protein and L2 capsid proteins, in an effective immunogenic concentration sufficient to induce a papillomavirus neutralizing antibody titer of at least about 10^3 when administered according to an immunizing dosage schedule.

28. The vaccine of Claim 27, wherein said L1 capsid protein is an HPV16 capsid protein.

29. The vaccine of Claim 28, wherein said L1 capsid protein is a wild type HPV16 L1 protein.

30. A method of preventing or treating papillomavirus infection in a vertebrate, comprising the administration of a papillomavirus structure according to Claim 18 or 19 to said vertebrate, according to an immunity-producing regimen.

31. The method of Claim 30 wherein said papillomavirus structure comprises wild type HPV16 L1 capsid protein.

32. A method of preventing or treating papillomavirus infection in a vertebrate, comprising the administration of the papillomavirus structure according to Claim 30 to said vertebrate, according to an immunity-producing regimen.

33. A method of preventing or treating papillomavirus infection in a vertebrate, comprising the administration of the papillomavirus vaccine of Claim 27 to said vertebrate, according to an immunity-producing regimen.

34. The method of Claim 33 wherein said papillomavirus vaccine comprises wild type HPV16 L1 capsid protein.

35. A method for immunizing a vertebrate against papillomavirus infection, comprising administering to said vertebrate a recombinant genetic construct comprising a conformational papillomavirus L1 coding sequence, and allowing said coding sequence to be expressed in the cells or tissues of said vertebrate, whereby an effective, neutralizing, immune response to papillomavirus is induced.

36. A method according to Claim 35, wherein said conformational papillomavirus L1 coding sequence is derived from human papillomavirus HPV16.

37. The method of Claim 36, wherein said human papillomavirus HPV16 is a wild type papillomavirus.

-34-

38. A method of detecting humoral immunity to papillomavirus infection in a vertebrate comprising the steps of:

- 5 (a) providing an effective antibody-detecting amount of a papillomavirus capsid peptide having at least one conformational epitope of a papillomavirus capsomer structure;
- (b) contacting the peptide of step (a) with a sample of bodily fluid from a vertebrate to be examined for papillomavirus infection, and allowing papillomavirus antibodies contained in said sample to bind thereto, forming antigen-antibody complexes;
- 10 (c) separating said complexes from unbound substances;
- (d) contacting the complexes of step (c) with a detectably labelled immunoglobulin-binding agent; and
- (e) detecting anti-papillomavirus antibodies in said sample by means of the labelled immunoglobulin-binding agent that binds to said complexes.

15 39. The method of Claim 37, wherein said peptide consists essentially of papillomavirus L1 capsid protein.

40. The method of Claim 38, wherein said peptide consists essentially of the expression product of a human papillomavirus HPV16.

20 41. The method of Claim 39, wherein said peptide consists essentially of the expression product of a wild type human papillomavirus HPV16 gene.

42. The method of Claim 40, wherein said peptide consists essentially of the expression product of SEQ ID NO:6.

43. A method of detecting papillomavirus in a specimen from an animal suspected of being infected with said virus, comprising

25 contacting said specimen with antibodies having a specificity to one or more conformational epitopes of the capsid of said papillomavirus, said antibodies having a detectable signal producing label, or being attached to a detectably labelled reagent;

allowing said antibodies to bind to said papillomavirus; and

30 determining the presence of papillomavirus present in said specimen by means of said detectable label.

44. A method of determining a cellular immune response to papillomavirus in an animal suspected of being infected with said virus, comprising;

-35-

contacting immunocompetent cells of said animal with a recombinant wild type papillomavirus L1 capsid protein, or combined L1 and L2 capsid protein according to Claim 18 or 19; and

5 assessing cellular immunity to said papillomavirus by means of the proliferative response of said cells to said capsid protein.

45. The method of Claim 44, wherein said recombinant papillomavirus protein is introduced into the skin of said animal.

46. A papillomavirus infection diagnostic kit, comprising capsomer structures consisting essentially of papillomavirus L1 capsid protein, or capsomer structures comprising
10 papillomavirus L1 protein and L2 capsid proteins, or antibodies to either of said capsomers structures, singly or in combination, together with materials for carrying out an assay for humoral or cellular immunity against papillomavirus, in a unit package container.

INTERNATIONAL SEARCH REPORT

Int. onal Application No
PCT/US 93/08342

A. CLASSIFICATION OF SUBJECT MATTER

IPC 5 C12N15/37 C12N7/04 C12N15/86 C12N5/10 A61K39/12
G01N33/53 C07K13/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 5 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA. vol. 89, no. 24 , December 1992 , WASHINGTON US pages 12180 - 12184 KIRNBAUER, R. ET AL. 'Papillomavirus L1 major capsid protein self-assembles into virus-like particles that are highly immunogenic' see the whole document --- -/--	1-46



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- *&* document member of the same patent family

Date of the actual completion of the international search

17 December 1993

Date of mailing of the international search report

18 -01- 1994

Name and mailing address of the ISA

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Fax (+31-70) 340-3016

Authorized officer

Chambonnet, F

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	<p>JOURNAL OF VIROLOGY vol. 67, no. 4 , April 1993 pages 1936 - 1944 ROSE, R.C. ET AL. 'Expression of human papillomavirus type 11 L1 protein in insect cells in-vivo and in-vitro assembly of viruslike particles' see the whole document ---</p>	<p>1-3,6-8, 10, 13-16, 18,21, 27,30, 32,33, 38,39, 43,46</p>
X	<p>JOURNAL OF GENERAL VIROLOGY vol. 72, no. 12 , December 1991 pages 2981 - 2988 XI, S.Z. & BANKS, L.M. 'Baculovirus expression of the human papillomavirus type 16 capsid proteins detection of L1-L2 protein complexes' see the whole document ---</p>	<p>1-3,5,6, 9-11,13, 16,19,20</p>
X	<p>VIROLOGY vol. 185, no. 1 , November 1991 pages 251 - 257 ZHOU, J. ET AL. 'Expression of vaccinia recombinant HPV16 L1 and L2 ORF proteins in epithelial cells is sufficient for assembly of HPV virion-like particles' see the whole document ---</p>	<p>7-11, 18-25, 27-37</p>
P,X	<p>WO,A,93 02184 (THE UNIVERSITY OF QUEENSLAND) 4 February 1993 see the whole document -----</p>	<p>7-11, 18-46</p>

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9302184	04-02-93	NONE	